

REMARKS

Claims 1, 16, 18, 20, 21, 33, 36, 39, 42 and 45 have been amended and new claims 49-51 have been added. Claims 1-22 and 33-51 are pending. Applicant submits that the amendment is fully supported by the specification and no new matter is introduced by the amendment.

Pursuant to the Examiner's inquiry in the Office Action, Applicant submits that Nature Medicine 4:1-5 (April, 1997) does not refer to an article by Guo et al. The correct citation is Guo et al., Nature Medicine vol. 3:451-455 (April 1997), which was submitted to the Examiner on January 9, 1998 with a Supplemental Information Disclosure Statement. Applicant's amendment dated February 9, 1998 corrected this typographical error in the specification.

The present invention relates to cellular vaccines and methods of preparation and use thereof. For example, claim 1 relates to a cellular vaccine comprising an **isolated autologous diseased cell** (e.g., tumor cell) (i) having enhanced level of primary and costimulatory T cell activation molecules and (ii) armed with a bridge molecule targeting one or more costimulatory molecules on the surface of T cells. In another example, claim 36 relates to a method of preparing and using such a cellular vaccine by (i) treating an isolated autologous diseased cell to increase the level of primary and costimulatory T cell activation molecules in the cell, (ii) arming the cell with a bridge molecule that targets one or more costimulatory molecules on the surface of T cells, (iii) collecting a pharmaceutically effective amount of treated cells and administering them to the patient from whom the cell was isolated. In dependent claims, the diseased cell is treated with cytokines such as IFN γ , TNF α , or both, and armed with a bridge molecule such as a bi-specific monoclonal antibody (Bi-Mab), e.g., CD28:gp55.

Once the primary and/or costimulatory T cell activation signals in the target diseased cells have been amplified by cytokines or other means and the bridge molecules have been attached to the target diseased cells, the cytokines and the bridge molecules not attached to the target diseased cells may be removed from the immunogenic composition before the target diseased cells are administered to a patient. This additional step minimizes adverse effects associated with administering cytokines to a patient. It also minimizes the risk associated with allowing bridge molecules not attached to a target diseased cell into a patient, an event which may cause unwanted immune response against normal or healthy cells.

See page 6 of the specification.

I. The Rejections under 35 U.S.C. §112, First Paragraph

A. Enablement for other materially different CD28:gp55 bispecific antibodies

The Examiner acknowledged that the specification is enabling for the compositions and methods of Example 6.6. However, the Examiner rejected claims 1-22 and 33-48 under 35 U.S.C. §112, First Paragraph, as not enabling for other materially different CD28:gp55 bispecific antibodies. The Examiner stated that:

Different monoclonal antibodies would be expected to bind to distinct epitopes of CD28 or gp55 and induce functionally distinct responses. For instance, a monoclonal antibody that binds to a portion of CD28 not involved in coactivation would not be expected to coactivate anti-tumor T cells.

Applicant respectfully traverses this rejection.

1. The specification teaches how to produce and select functional anti-CD28 antibodies

The specification describes a process of producing and selecting anti-CD28 antibodies for this invention (e.g., see Example 6.2, page 32).

Anti-mouse CD28 Mabs were generated by first immunizing Wistar rats with a mouse T cell hybridoma cell line expressing high levels of CD28 antigen on the cell surface. After cell fusion, hybridomas producing anti-CD28 Mab were selected with immunofluorescent analysis by FACScan. Anti-CD28 Mabs were further characterized and confirmed by immunoprecipitation and T cell proliferation and IL-2 production assays. Example 6.2 further describes that each of the Bi-Mabs was tested both *in vitro* and *in vivo* for its ability in combination with cytokine treatment to activate T cell response against the target diseased cells using CTL toxicity, tumor growth, and animal survival assays (*see* Examples 6.3-6.6).

2. There is no need to describe standard and routine techniques

Applicant directs those skilled in the art to characterize Mabs with standard and routine immunology and cell biology techniques such as immunoprecipitation, T cell proliferation, IL-2 production, CTL toxicity, tumor growth, and animal survival assays. Since a patent need not teach and preferably omits, what is well known in the art, Applicant submits that the specification has enabled the use of different CD28:gp55 bispecific antibodies in relation to claims 1-22 and 33-48 by teaching how to produce and select functional Mabs. *See, Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986).

3. Enablement does not require all possible species to be tested

It is not necessary for the enablement of claims 1-22 and 33-48 to discover and test all of the possible anti-CD28 Mabs in the world. For enablement of a generic claim, there is no requirement that every species must be tested. Such a requirement would be against the policy of the patent laws:

such a requirement would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments. This would tend to discourage inventors from filing patent applications in an unpredictable area since the patent claims would have to be limited to those embodiments which are expressly disclosed.

In re Angstadt and Griffin, 190 U.S.P.Q. 214, 218 (CCPA, 1976).

In a situation analogous to that presented here, the PTO Board of Patent Appeals and Interferences (the "Board") reversed an enablement rejection and held that a claim directed to proteins generally was enabled when the specification only disclosed specific examples. Ex parte Mark, 12 U.S.P.Q. 1904 (PTO Bd.Pat.App. & Int. 1989). The methods and products in Mark involved "muteins," described as proteins in which cysteines not essential for biological activity are substituted with another moiety. The Examiner rejected the claims because the specification disclosed only specific examples (IFN- β , IL-2, and TNF) and the claims at issue read on both operative and inoperative species. The Board reversed the examiner's rejection.

The Board pointed out that one skilled in the art would be able to routinely determine if such cysteine substitution would be deleterious to biological activity. Thus, the Board reasoned, undue experimentation would not be required for one skilled in the art to practice the claimed invention for a given protein:

The fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement. One skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity.

12 U.S.P.Q. at 1907. Moreover, with regard to as yet-undiscovered proteins and polypeptides, the Board in Mark held that it would be within the skill of the art to test such proteins to see if they retained biological activity after cysteine substitution.

Experimentation is not inconsistent with enablement, providing that it is not undue. Thus, the fact that experimentation may be complex, as testified to in this case, does not necessarily make it undue, if the art typically engages in such experimentation.

In re Certain Limited-Charge Cell Culture Microcarriers, 221 U.S.P.Q.1165, 1174 (USITC 1983).

Given the routine and standard assays taught in the specification of this patent application, one skilled in the art is clearly enabled to performed such work as needed to determine whether a given anti-CD28 Mab is able to stimulate T cell response.

Therefore, Applicant respectfully submits that the specification describes to those skilled in the art how to prepare and select effective CD28:gp55 bispecific antibodies and this rejection under 35 U.S.C. §112, first paragraph be withdrawn.

B. Enablement for treatment of cancer cells not comprising gp55

The Examiner rejected claims 1-22 and 33-48 under 35 U.S.C. §112, First Paragraph, as not enabling for treatment of cancer cells not comprising gp55. The Examiner stated that:

Tumor cells which lack the gp55 determinant would not be targeted by antibodies which bind to gp55. Methods of using bispecific antibodies comprising determinants which bind to gp55 would not be expected to target and destroy tumor cells comprising non-gp55 antigens.

Applicant respectfully traverses this rejection.

Applicant respectfully submits that the specification not only describes treating tumor cells comprising gp55, but also describes treating tumor cells comprising other antigens. CD28:gp55 is but one species encompassed by claims 1-22 and 33-48.

The specification describes targeting antigens other than gp55 on tumor cells. For example, Example 6.2 describes bispecific antibodies targeting gp95 and gp210 in addition to gp55. gp95 and gp210 are expressed on tumor cells. Fig. 2 of the specification indicates that the bispecific antibodies against gp95 and gp210 stimulated splenic T cell proliferation. Example 6.6 describes that hepatomas in the mice treated with the CD28:gp55, CD28:gp95 and CD28:gp210 Bi-Mabs regressed to undetectable size within about 40 days (Fig. 6). Pages 10-11 of the specification describe other antigens on the target cell suitable for anchoring the bridge molecule.

The specification also describes a process of identifying antigens on target cell surface and antibodies that bind to the target cells by immunofluorescent staining (*see e.g.* Example 6.2). This process identified gp55, gp95 and gp210 on the surface of hepa 1-6 cells.

Therefore, Applicant respectfully submits that the specification enables those skilled in the art to treat diseased cells not comprising gp55 and this rejection under 35 U.S.C. §112, first paragraph be withdrawn.

C. Enablement in relation to receptors for IFN γ and TNF

The Examiner rejected claims 1-22 and 33-48 under 35 U.S.C. §112, First Paragraph, as not enabling for treatment of cancer cells not comprising receptors for IFN γ or TNF. The Examiner stated that:

Tumor cells that do not express receptors for IFN γ or TNF would not be expected to react to the presence of these mediators. Paul indicates that cytokines must bind to specific cellular receptors in order to exert a biological effect.

Applicant respectfully traverses this rejection.

Applicant respectfully submits that both IFN γ and TNF are widely expressed in different types of cells, including tumor cells.

IFN γ exerts its pleiotropic effects on cells through an interaction with a specific receptor expressed at the cell surface. On the basis of immunochemical, radioligand binding, and molecular genetic analysis, there appears to be only a single type of IFN γ receptor that is ubiquitously expressed on all cells (except the erythrocyte).

See page 582 of Farrar, Annu. Rev. Immunol. 1993, 11:571-611, a copy of which is enclosed for the Examiner's reference.

"TNF receptors are expressed on nearly all nucleated cell types." See page 202 of Rink and Kirchner, Int. Arch. Allergy Immunol. 1996, 111:199-209 (not admitted to be prior art, a copy is enclosed for the Examiner's reference).

More importantly, the specification not only describes using IFN γ or TNF to enhance the level of primary and costimulatory T cell activation molecules in target diseased cells, but also describes using other means to stimulate the expression of primary and costimulatory T cell activation molecules in the target cells, including using other cytokines. IFN γ and TNF are but two species encompassed by claims 1-22 and 33-48. For example, pages 14 and 15 of the specification describe that IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, inteferons (e.g., IFN α , β , and γ), tumor necrosis factors (e.g., TNF α , and β) and other chemokines and lymphokines can be used to enhance the expression of primary and costimulatory T cell activation molecules.

Therefore, Applicant respectfully submits that the specification enables those skilled in the art to treat cancer cells not comprising receptors for IFN γ or TNF and this rejection under 35 U.S.C. §112, first paragraph be withdrawn.

II. The Rejection under 35 U.S.C. §103(a)

The Examiner rejected claims 1-22 and 33-48 under 35 U.S.C. §103(a) as allegedly unpatentable over Li, Renner, or Krummel in view of Paul and Darlington. However, given the differences between the present invention and the references cited by the Examiner, the references fail to support a *prima facie* case of obviousness. Applicant respectfully traverses this rejection.

A. The references cited by the Examiner

Li transfected murine melanoma cells with a human tumor-associated antigen, p97, and the murine B7 gene, and co-expressed the two genes in the murine melanoma cells. Li then injected the transfected melanoma cells into mice and showed that they increased systemic anti-tumor immunity via activation of both CD4⁺ and CD8⁺ T cells.

Renner prepared Bi-Mabs that bound (i) to a Hodgkin's tumor-associated antigen (CD30) on the tumor and (ii) to either CD3 or CD28 on the T cell. Renner then injected the Bi-Mabs to immuno-deficient mice implanted with human Hodgkin's derived tumor. Renner also injected to the same mice human peripheral blood lymphocytes that had been pre-stimulated by incubation with the CD3-CD30 Bi-Mab and cells that expressed CD30. The established tumor was cured by the double injections of Bi-Mabs and pre-stimulated human peripheral blood lymphocytes. Renner also mentioned that T cells could be activated by IL-2 and anti-CD3 antibody.

Krummel described that "CD28 engagement via antibodies augments the proliferation of T cells in response to immobilized anti-TCR antibodies." See page 459 of Krummel. Krummel prepared an antibody to CTLA-4 molecule on T cells and described that CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation.

Paul (p815-p826) describes that IFN γ enhances antigen presenting function of macrophages and that IFN γ have been used directly to treat diseases. Paul also describes the activities of TGF, but not that of TNF.

Darlington describes the establishment of a mouse hepatoma cell line, Hepa, and its subclone, Hepa-1.

The Examiner argued that it would have been obvious to make Bi-Mabs binding to CD28 on T cells and binding to antigens on tumor cells. The Examiner also argued that the primary references (i.e., Li, Renner and Krummel) teach that such Bi-Mabs would provide costimulation to CTLs and thus enhance anti-tumor CTL activity. Furthermore, the Examiner argued that the addition of IFN- γ and TNF would activate antigen-presenting cells (APC) like macrophages, augment T cell responses and other cellular responses such as increases in neutrophil adhesion useful for targeting or destroying tumor cells.

B. Burden of proof by the Examiner

The examiner bears the burden of establishing a *prima facie* case of obviousness. Only if this burden is met does the burden of coming forward with rebuttal argument or evidence shift to the applicant. When the references cited by the examiner fail to establish a *prima facie* case of obviousness, the rejection is improper and will be overturned. (citations omitted) In re Deuel, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995).

As discussed below, the references cited by the examiner fail to establish a *prima facie* case of obviousness.

C. Differences between the references cited by the Examiner and the claimed invention

When evaluating a claim for determining obviousness, all limitations of the claim must be evaluated. The invention must be reviewed as a whole. In re Gulack, 217 USPQ 401 (Fed. Cir.

1983). In that regard, the Examiner's emphasis on the alleged obviousness of making bi-specific monoclonal antibodies binding to CD28 on T cells and binding to antigens on tumor cells is misplaced because the invention as a whole is directed to cellular vaccine comprising an isolated autologous diseased cell as described above.

The Examiner's emphasis on the activities of IFN γ and TNF on antigen-presenting cells such as macrophages is also misplaced because the claimed invention relates to using IFN γ and TNF to stimulate the isolated autologous diseased cell.

The following comparison details the differences between the claimed invention and the references cited by the Examiner.

1. Li did not arm tumor cells with a bridge molecule

Li injected melanoma cells expressing p97 and B7 genes into mice. Li does not describe or suggest attaching a bridge molecule such as a Bi-Mab to the melanoma cells and using the bridge molecule to target one or more costimulatory molecules on the surface of T cells. In addition, the Examiner did not provide any evidence that the CD28:CD30 and CD3:CD30 Bi-Mabs of Renner would bind to the melanoma cell of Li.

2. Renner teaches away from administering isolated autologous target diseased cell to the host

The claimed invention is directed to cellular vaccines containing isolated autologous target diseased cell armed with bridge molecule and to administering isolated autologous target diseased cell back to its host after treatment of the cell to increase its immunogenicity. Instead of administering an isolated autologous target diseased cell, however, Renner injected to the host (i) two Bi-Mabs, and (ii) a population of heterologous blood lymphocytes.

In another aspect of differences between the present invention and Renner, the claimed invention uses cytokines such as IFN γ and TNF to enhance the level of primary and costimulatory T cell activation molecules in the isolated autologous target diseased cell while Renner described using a cytokine such as IL-2 or anti-CD3 antibody to activate T cells.

3. Krummel did not use Bi-Mab or isolated autologous target diseased cell

In comparison to the claimed invention, Krummel did not isolate any autologous target diseased cell to prepare a cellular vaccine or prepare or use any bridge molecule such as Bi-Mab to arm an isolated autologous target diseased cell. Furthermore, the T cell response described by Krummel as augmented by the engagement of CD28 with antibodies is in relationship to immobilized anti-TCR antibodies, which is different from T cell response to an autologous target diseased cell.

4. Paul did not describe using IFN γ or TNF to increase the level of primary and costimulatory T cell activation molecules in an isolated autologous target diseased cell

This invention relates to using IFN γ and TNF to enhance the level of primary and costimulatory T cell activation molecules in an isolated autologous target diseased cell such as an isolated hepatoma cell. Paul, however, only describes using IFN γ to enhance antigen-presenting function of normal macrophages. Furthermore, Paul in pages 815-826 does not describe whether TNF can enhance the level of primary and costimulatory T cell activation molecules in an isolated autologous target diseased cell.

5. Darlington does not make up for the deficiencies in the primary references

None of the deficiencies in the primary references is made up by Darlington, which describes the establishment of a mouse hepatoma cell line and its subclone.

The above analysis shows that the claimed invention is different from the references cited by the Examiner in so many ways that it is not described or suggested by these references either alone or in combination.

D. The cited references provide no suggestion or motivation to make the claimed invention

Where one element of the claimed invention is found in one reference, and another element of the claimed invention is found in another reference, the teachings of the two references can be combined **only if** there is some suggestion or incentive to do so. In re Fine, 5 U.S.P.Q.2d 1596, 1599 (Fed. Cir. 1988). In addition, the motivation or suggestion for combining the teaching must be other than the knowledge learned from the disclosure of the applicant. In re Laskowski, 10 U.S.P.Q.2d 1397, 1398 (Fed. Cir. 1989). In this case, no suggestion or motivation was given in the references cited by the examiner.

Not until after the provisional application 60/019,639 was filed on June 12, 1996 were there publications that described preparing cellular vaccines by arming an isolated autologous target diseased cell with a bridge molecule that target one or more costimulatory molecules on the surface of T cells and increasing the level of primary and costimulatory T cell activation molecules in the cell, e.g., Guo et al., Nature Medicine vol. 3:451-455 (April 1997).

The only way the disclosures of Li, Renner, Krummel, Paul and Darlington can be read to result in the claimed invention is with benefit of applicant's disclosure. Such use of applicant's disclosure is improper. Selective hindsight cannot be used to evaluate obviousness. There must be a reason or suggestion in the prior art for selecting the materials and procedure used, other than the knowledge learned from the applicant's disclosure. In re Dow Chem. Co., 5 U.S.P.Q.2d 1529, 1532 (Fed. Cir. 1988).

A patentable invention may lie in the discovery of the source of a problem even though the remedy may be obvious once the source of the problem is identified. This is part of the "subject matter as a whole" which should always be considered in determining the obviousness of an invention under 35 U.S.C. § 103.


In re Peehs, 204 USPQ 835, 837 (CCPA, 1980).

Accordingly, the claims are now in condition for allowance and a notice to that effect is respectfully requested. If there is any fee due in connection with this response, please charge Deposit Account No. 12-2475 for the appropriate amount.

Respectfully submitted,

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